



3058 Research Drive
State College, Pennsylvania 16801 USA
Telephone: 814.272.1039
Fax: 814.272.1019

Analytical Report

PFOA and PFOS Analysis of Deer Muscle and Liver Samples by LC/MS/MS

MPI Report No. L0019346

Revised Report Date: 12/17/09

Testing Laboratory

MPI Research, Inc.
3058 Research Drive
State College, PA 16801

Requester/Project Manager

Dena Haverland
Dalton Utilities
PO BOX 869
Dalton, GA 30722
Phone: 706-529-1010

2009 JUN 9 P 1:14u

1 Introduction

Results are reported for the analysis of the 3.5 yr male deer liver sample received at MPI Research from Dalton Utilities. The MPI Research study number assigned to the project is L0019346. Table I lists the target analytes quantitated for the samples.

Table I. Target Analytes for Quantitation

Compound Name	Acronym
Perfluorooctanesulfonate	C8 Sulfonate or PFOS

Note: PFOA and PFOS results for all the muscle and liver samples with the exception of the 3.5yr male deer liver sample are reported in the original report signed on 11/19/09.

2 Sample Receipt

Four samples were received from Dena Haverland at Dalton Utilities for this study. The samples were collected on October 02, 2009. The samples arrived on October 06, 2009 via Fedex and were logged in under MPI Research login number L0019346. The shipment was received frozen on dry ice. The samples were stored frozen at approximately -20°C from receipt until analysis. Chain-of-custody information is presented in Attachment A.

3 Methods - Analytical and Preparatory

3.1 Muscle and Liver Sample Preparation

- 3.1.1. Weigh 1 g of muscle or liver sample into a 50 mL disposable centrifuge tube and fortify, if appropriate. Add 20 µL of a 50000 ng/mL WIS for a final concentration of 1 ng/mL.

Note: The internal standard was spiked at a higher level to allow for post extraction dilutions to be performed.

- 3.1.2. Add water to the sample for a final volume of 10 mL. Cap tightly.
- 3.1.3. Homogenize sample using a tissuemizer for ~1 minute.
- 3.1.4. Transfer 1 mL of the sample using a disposable pipette into 15 mL disposable centrifuge tubes. Add 5 mL of ACN and shake for ~20 minutes on a wrist action shaker.
- 3.1.5. Centrifuge tubes at ~3000 rpm for ~ 5 minutes. Carefully decant supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
- 3.1.6. Place the unconditioned SPE columns on the vacuum manifold. Condition the SPE columns by passing ~ 10 mL of methanol through the column followed by ~ 5 mL of water. The washes may be pulled through the SPE column using vacuum at a flow rate of ~1 drop/sec or may be allowed to pass through the column unaided. Discard all washes. Do not allow the column to dry.

- 3.1.7 Load the sample onto a conditioned SPE column . Discard the eluate. Any analyte residues will be trapped on the SPE column at this point.
- 3.1.8 Elute with 2 mL of methanol. Collect 2 mL of elute into a graduated 15 mL centrifuge tube.

Note: Post extraction dilutions were prepared in methanol.

3.2 Sample Analysis by LC/MS/MS

In High Pressure Liquid Chromatography (HPLC), an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, mass spectrometry provides a rapid and accurate means for analyzing a wide range of organic compounds. Molecules are ionized, fragmented, and detected. The ions characteristic of the compounds are observed and quantitated against external calibration standards.

An HP1100 system interfaced to an Applied Biosystems API 4000 LC/MS/MS was used to analyze the sample extracts for quantitation. A gradient elution through a Phenomenex Luna 3 μ C8(2) Mercury, 20 x 4.0 mm column was used for separation.

The following gradient was performed:

Mobile Phase (A): 2mM Ammonium Acetate in Water
Mobile Phase (B): Methanol

Time	%A	%B
0.0	90	10
0.5	90	10
2.0	10	90
5.0	10	90
5.1	0	100
6.0	0	100
6.1	90	10
10.0	90	10

The following parameters were used for operation of the mass spectrometer:

Parameter	Setting
Ionization Mode	Electrospray
Polarity	Negative
Transitions Monitored	499 \rightarrow 80 (PFOS) 503 \rightarrow 80 (Internal Std. ^{13}C PFOS ($m+4$))
Gas Temperature	450°C

4 Analysis by LCMSMS

4.1 Calibration

For the muscle and liver sample analysis, a 6-point calibration curve was analyzed throughout the analytical sequence for PFOS. The calibration points were prepared at 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 ng/mL (ppb) containing 1.0 ng/mL ^{13}C -PFOS (m+4).

The ratio of the analyte concentration to the IS concentration versus the ratio of the analyte instrument response (area) to the IS response (area) was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r^2 \geq 0.985$.

For the results reported here, calibration criteria were met. The calibration curves are included in the raw data in Attachment C.

4.2 Laboratory Control Spikes

Laboratory control spikes in the analytical set were prepared during each extraction set by adding a known concentration of the analyte to deer muscle and liver controls. Laboratory control spikes are used to assess method accuracy. The laboratory control spikes must show recoveries between 70-130% or the data is rejected. For the results reported here, the laboratory control spikes were within the acceptable range. Laboratory control spike recoveries are given in Attachment B.

4.3 Matrix Spikes

A matrix spike was prepared for each sample by adding a known concentration of the target analyte to a sample. Matrix spikes are used to assess method accuracy in the matrix. The matrix spikes should show recoveries between 70-130%. For the results reported here, the matrix spike was within the acceptable range with the exceptions of:

4.4 Laboratory Duplicates

Each sample was prepared in duplicate and analyzed. Duplicate results are given along with the sample results in Attachment B.

5 Data Summary

Please see Attachment B for a detailed listing of the analytical results. For the muscle and liver samples the results are reported in parts per billion (ng/g) on an as-received basis.

6 Data/Sample Retention

Samples are disposed of 60 days after the report is issued unless otherwise specified by the project manager. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by MPI Research. Hardcopy data is stored for a minimum of five years. The client will be notified 30 days prior to the disposal of hardcopy data.

7 Attachments

- 7.1 Attachment A: Chain of Custody
- 7.2 Attachment B: Analytical Results
- 7.3 Attachment C: Raw Analytical Data for Water

8 Signatures



Mark Neelley, Research Chemist Associate II

12-17-09
Date



Robert Zhu, Manager, Analytical

12/17/09
Date

A

Mattawan (Corporate Headquarters)
 54943 North Main Street
 Mattawan, MI 49071-9399
 (269) 668-3336 Phone
 (269) 668-4151 Fax

State College
 3058 Research Drive
 State College, PA 16801
 (814) 272-1039 Phone
 (814) 231-1580 Fax

Login

Login Group: L0019346

Login #:	19460	Conform COC Sample:	True
Project:	P0005196	Conform COC:	True
Company Name:	Dalton Utilities	Conform Sample:	True
Submitted By:	Dena Haverland	Conform Request:	True
Login Type:	Immediate Receipt of Samples		
Started:	True		
Date Start:	10/27/2009		
Due Date:	11/06/2009		
Login Initiated:	10/27/2009		
Received By:	Ammerman, Mark		
Spread Sample:			
Label:			
MPI SD/PI:	Zhu, Xiang		

Project Title/Type: PFOA and PFOS Analysis of Animal Muscle and Liver by LC/MS/MS / ROUTINE

Login Notes:

Packages / Containers

<u>Package</u>	<u>Carton</u>	<u>Date / Condition</u>		<u>Shipper / ID</u>	<u>Temp. Control/Temp.</u>	<u>Direction / Handled By</u>
K0022042		Received Date: 10/27/09 10:25 Package & Contents Uncompromised		FEDEX 8694 2057 8178	Dry Ice -79.2	RECEIVED Ammerman, Mark
Container #	Gross Weight	pH	Container Type	Preservative	Mfg. Lot	Mfg. ID
C0457624	218.20 g		1/2 gallon ziplock bag	NONE		
C0457625	278.00 g		1/2 gallon ziplock bag	NONE		
C0457626	324.10 g		1 gallon ziploc bag	NONE		
C0457627	994.10 g		1 gallon ziploc bag	NONE		

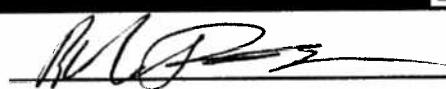
Samples

<u>Sample ID</u>	<u>Container</u>	<u>Matrix</u>	<u>System</u>	<u>System Matrix</u>	<u>Sample</u>	<u>Date Sampled</u>	<u>Date Due</u>
L0019346-0001	C0457624	SOLID	Deer	Tissue	Deer #6 0.5 yr female-muscle	10/02/2009	11/06/2009
L0019346-0002	C0457626	SOLID	Deer	Liver	Deer #6 0.5 yr female-liver	10/02/2009	11/06/2009
L0019346-0003	C0457625	SOLID	Deer	Tissue	Deer #7 3.5 yr male-muscle	10/02/2009	11/06/2009
L0019346-0004	C0457627	SOLID	Deer	Liver	Deer #7 3.5 yr male-liver	10/02/2009	11/06/2009



Login

Login Reviewed By:



Date/Time:

12/16/09 13:17



MPI

RESEARCH

Sample Submittal

MPI Research Contact: Daniel Wright

Send Report To:	
Company: Dalton Utilities 1200 VD Parrott JK Parkway, PO Box 869	
Address: _____	
City, State, ZIP: Dalton, GA 30722-0869	
Attention: Dena Haverland	
Phone #: 706-529-1010	
Fax #: 706-529-1271	
Email: dhaverland@dutil.com	
Study/Job #: _____	
Signature/Date: _____	
Printed Name: _____	

Please fax this form before sending samples.	
Please send samples to shipping and receiving: 3048 Research Drive, State College, PA 16801 T: (814) 272-1039 • F: (814) 272-1019	
Turnaround time (TAT) requirements: Results Due Date: <u>30 days</u>	
Preliminary Results Format: Verbal <input checked="" type="checkbox"/> Email <input type="checkbox"/> Fax	
Report Due Date: <u>30 days</u>	
Storage Conditions Room temperature <input checked="" type="checkbox"/> Refrigerator <input checked="" type="checkbox"/> Freezer <input checked="" type="checkbox"/> Ultra Low freezer <input checked="" type="checkbox"/> Desiccated <input checked="" type="checkbox"/> Lighting required	Safety Information Special handling: _____ <input checked="" type="checkbox"/> MSDS attached Controlled substance: _____ HAZARDS: _____
Stability time period: _____	

	Client ID# Description	Lot/ Control #	Amt. Sent/ Weight	# of Bottles	Matrix	Date & Time	Tests Requested
1	Deer #6 0.5 yr female - serum		1.0 ml	10	deer	10/2/09 1:08AM	PFCA/PFOS
2	Deer #6 0.5 yr female - muscle		1.0 mg	1 bag	deer	10/2/09 2:28AM	PFCA/PFOS
3	Deer #6 0.5 yr female - Liver		1.0 mg	1 bag	deer	10/2/09 2:36AM	PFCA/PFOS
4	Deer #7 3.5 yr Male - Serum		1.0 ml	10	deer	10/2/09 1:45AM	PFCA/PFOS
5	Deer #7 3.5 yr Male - Muscle		1.0 mg	1 bag	deer	10/2/09 2:45AM	PFCA/PFOS
6	Deer #7 3.5 yr Male - Liver		1.0 mg	1 bag	deer	10/2/09 2:48AM	PFCA/PFOS
7							
8							
9							
10							

PO #

Renlinished by: Dan Date: 10/5/09 Received by: Dan Date: 10/5/09
Daniel Wright 10/5/09 6:35AM 10/5/09

Notes

'THIS IS AN EXACT COPY OF
THE ORIGINAL DOCUMENT'
BY MIA DATE 10/27/09



TEMPORARY SAMPLE STORAGE FORM

To be completed during ExyLIMS Login

Project #: 15196

Login #: L4346

Initials / Date: AC 10/27/09

One form to be completed for each package

Date / Time Received: 10/26/09 1025

Received By: Mark Ammons

Shipper: FedEx

Shipper Package ID: 8694 2057 8178

Temperature (deg C) / Thermometer ID: -79.2 / D0001771

Temperature Control Method: dry ice-active

Temporary Storage Location: Walkin Freezer II

Condition of sample(s):

- Good - Package and contents uncompromised
- Fair - Package damaged / contents uncompromised
- Poor - Package and contents compromised

Notes:



3058 Research Drive
State College, PA 16801
Ph.: 814-272-1039
Fax: 814-231-1580

SAMPLE PROCESSING RECORD

Processing Requested By Employee: Mark Neeley

MPI Research Assigned Project Number: P0005196

MPI Research Assigned Login Number: L0019346

Date Processed: 10/29/2009 Time Started: 14:57 Time Finished: 17:20

Processed By Employee/Employees: Eric Edwards

Processing Instructions/Comments:

B



3058 Research Drive
State College, Pennsylvania 16801 USA
Telephone: 814.272.1039
Fax: 814.272.1019

Analytical Report

Summary of Fluorochemical Residues in Liver Samples

PFOS Perfluorooctanesulfonate	
Sample ID	Analyte Found (ng/g, ppb)
Deer # 7 3.5 yr male-liver	2750
Deer # 7 3.5 yr male-liver*	2600

*Laboratory Duplicate

ND = Not detected = Response is below the LOD of 1.0 ng/g (ppb).

NQ = Not quantifiable = Response is between the LOD and the LOQ of 10 ng/g (ppb).



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Recovery Summary of Fluorochemical Residues in Liver Samples

PFOS				
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)
LCS A (Data set 112409B) 2000 ng/g	2000	16.5	2340	116
LCS B (Data set 112409B) 2000 ng/g	2000	16.5	2170	108
Deer # 7 3.5 yr male-liver (L19346-4 Spk C, 2000 ng/g Lab Spike)	2000	2750	4770	101

ND = Not detected = Response is below the LOD of 1.0 ng/g.

NQ = Not quantifiable = Response is between the LOD and the LOQ of 10 ng/g.

C

RAW DATA REPORT

Sponsor Study No: NA Limit of Quantitation: 10 ng/g Set No: 112409B
 MPI Study No: L19346 Injection Volume: 15 µL Analyst: Mark Neely
 Analyte: PFOS Matrix: Deer Liver Instrument Type: LC/MS/MS Unit # 9
 Ions Monitored: 499 -> 80 Sample Weight 1.0 g Extraction Date: 11/24/09
 Site: NA Analyzed on: 11/24/09

MPI Research ID	Sponsor ID	Sample Code	Sample Index No.	Internal				Internal		Amount	
				Std. Conc. (ng/mL)	Std. Conc. (ng/mL)	Aliquot Factor (AF)	Dilution Factor (DF)	Peak Area	Standard Peak Area	Analyte Found (ng/g)	Analyte Added (ng/g)
SS33618	-	CS	1	0.100	1.0	-	-	34904	308667	-	-
SS33617	-	CS	2	0.200	1.0	-	-	67282	306260	-	-
SS33616	-	CS	3	0.500	1.0	-	-	162562	307963	-	-
SS33615	-	CS	4	1.00	1.0	-	-	335048	313198	-	-
SS33614	-	CS	5	2.00	1.0	-	-	663251	302152	-	-
SS33613	-	CS	6	5.00	1.0	-	-	1540686	295604	-	-
Methanol Wash	-	W	7	-	-	-	-	633	0	-	-
Methanol Wash	-	W	8	-	-	-	-	833	0	-	-
Control	MC4313 Deer Liver Control	C	9	-	1.0	20	1	221553	250243	16.5	-
LCS A	MC4313 Deer Liver Spike A, 2000 ng/g	LCS	10	-	0.5	20	100	248833	100052	2338	2000
LCS B	MC4313 Deer Liver Spike B, 2000 ng/g	LCS	11	-	0.5	20	100	278488	120785	2167	2000
L19346-4 Spk C	Deer # 7 3.5 yr male-liver Spike C, 2000 ng/g	LF	12	-	0.5	20	100	590437	116645	4767	2000
L19346-4	Deer # 7 3.5 yr male-liver	S	13	-	0.5	20	100	327125	111737	2754	-
L19346-4 Dup	Deer # 7 3.5 yr male-liver Duplicate	S	14	-	0.5	20	100	314680	113731	2602	-
SS33615	CCV, 1.0 ng/mL	CCV	15	1.00	1.0	1	1	324083	303718	1.00	1.00

Analyte Found (ng/g) = (((analyte peak area/IS peak area) - intercept) / slope) x IS conc. x AF x DF/Sample weight

Standard Curve : Linear (1/x weighted)

Recovery (%) =

$$\frac{[\text{Analyte found (ng/g)} - \text{Analyte found in control (ng/g)}]}{\text{amount Analyte added (ng/g)}} \times 100$$

Intercept = 0.00878

Slope = 1.06

Coef. Of Det. = 0.9994

CS = Calibration standard

LF = Lab fortified sample

W = Methanol Wash

CCV = Continuing Calibration Verification

FF = Field fortified sample

ND = Not detected = Response between 0 and LOD

C = Control sample

LCS = Laboratory Control Spike

S = Sample

NQ = Not quantifiable = Response between LOD and LOQ

Spreadsheet prepared by: M.D.H. 11-25-09



3058 Research Drive
State College, PA 16801

Phone: 814-272-1039
Fax: 814-231-1580

Internal Chain of Custody/Fortification Sheet

MPI Research Study Number:
Sponsor Study/Protocol No:

L19346

Matrix: Deer Liver

The samples listed below were removed from Freezer No. 36.

Time 9:42 am

Date 11-24-05

Initials MW

	Spiking Solution Used	Volume Used for Spiking	Initial/Date
MC4313 LCS A	5233971 (100000 ng/mL)	20 µL (5-100µL auto-pipette)	MDN 11-24-09
MC4313 LCS B	5533971 (100000 ng/mL)	20 µL (5-100µL auto-pipette)	MDN 11-24-09
L19346-4 Spk C	5533971 (100000 ng/mL)	20 µL (5-100µL auto-pipette)	MDN 11-24-09
MC4313 Control	SS0032652 (100 ng/mL)	200 µL (50-250µL auto-pipette)	MDN 11-24-09
All Samples (except control)	SP0011659 (50000 ng/mL)	20 µL (5-100µL auto-pipette)	MDN 11-24-09

All samples were weighed on balance 20

Time 9:43 AM

Date 1-24-09

Initials MJS

After weighing samples were returned to Freezer No. 36

Time 9:55 am

Date 11-24-69

Initials M.W.

Comments:

Analysis Summary:

Data Set: 112709B

Initials/Date: MSP / 11-27-09

Data Set: —

Initials/Date: — / —

Data Set: —

Initials/Date: /

Set extraction/analysis data verified by:

Date:

Nov 02, 2009/2

alyst Version: 1.4.2

rinting Time: 3:33:23 PM

rinting Date: Tuesday, November 24, 2009

MPI Study: L19346

MPI Set No.: 112409B

Operator: Mark Neeley

Instrument No.: LC/MS/MS #9

object: \\sc1wp5556\mdrive\PE SCIEX DATA\Projects\P5195 Batch:09_112409B Tab:Sample Set:SET1 AcqMethod:P5195_102909.d

Sample

MDP 11-24-09

Sample Name	Sample ID	Vial Position	Data File
SS33618	Calibration Standard, 0.1 ng/mL	11	09_112409B\112409B
SS33617	Calibration Standard, 0.2 ng/mL	12	09_112409B\112409B
SS33616	Calibration Standard, 0.5 ng/mL	13	09_112409B\112409B
SS33615	Calibration Standard, 1.0 ng/mL	14	09_112409B\112409B
SS33614	Calibration Standard, 2.0 ng/mL	15	09_112409B\112409B
SS33613	Calibration Standard, 5.0 ng/mL	16	09_112409B\112409B
Methanol Wash	Methanol Wash	91	09_112409B\112409B
Methanol Wash	Methanol Wash	91	09_112409B\112409B
Control	MC4313 Deer Liver Control	27	09_112409B\112409B
LCS A	MC4313 Deer Liver Spike A, 2000 ng/g	28	09_112409B\112409B
LCS B	MC4313 Deer Liver Spike B, 2000 ng/g	29	09_112409B\112409B
L19346-4 Spk C	Deer # 7 3.5 yr male-liver Spike C, 2000 ng/g, DF=100	30	09_112409B\112409B
L19346-4	Deer # 7 3.5 yr male-liver, DF=100	31	09_112409B\112409B
L19346-4 Dup	Deer # 7 3.5 yr male-liver Duplicate, DF=100	32	09_112409B\112409B
SS33615	CCV, 1.0 ng/mL	14	09_112409B\112409B

LC/MS/MS SYSTEM AND OPERATING CONDITIONS

Protocol No: NA

MPI Study No: L19346

Instrument: AB API 4000 Biomolecular Mass Analyzer, (LC/MS/MS #9)
SCIEX Turbo Ion Spray Liquid Introduction Interface
Turbo Ion spray temperature = 450 °C

Computer: Dell OptiPlex GX 110

Software: PE Sciex Analyst 1.4

HPLC Equipment: Hewlett Packard (HP) Series 1100
 HP Quat Pump HP Vacuum Degasser
 HP Autosampler HP Column Oven

HPLC Column: Phenomenex Luna C8 (2) Mercury, 2cm x 4mm, 3 µm (ExyLIMS ID:
MA0052622)

Column Temperature: 35°C

Mobile Phase (A): 2 mM Ammonium Acetate in Water (ExyLIMS ID: SL0045925)

Mobile Phase (B): Methanol (ExyLIMS ID: RE0047880)

Injected Volume: 15 µL

<u>Time (min)</u>	<u>% A</u>	<u>% B</u>	<u>Flow Rate (μL/min)</u>
0.0	90	10	750
0.5	90	10	750
2.0	10	90	750
5.0	10	90	750
5.1	0	100	750
6.0	0	100	750
6.1	90	10	750
10.0	90	10	750

Jons monitored:

<u>Analyte</u>	<u>Parent ion</u>	<u>Daughter ion(s)</u>	<u>Dwell (secs)</u>
PFOS	499	80	0.200
¹³ C PFOS (m+4)	503	80	0.200
Internal Standard			

Analyst:

Mark Neeley 11-24-09

MPI Research, Inc.

3058 Research Drive, State College, PA 16801

Phone: (814) 272-1039 FAX: (814) 231-1580

All Handwritten Peak ID's by: *MDB 11-25-09*

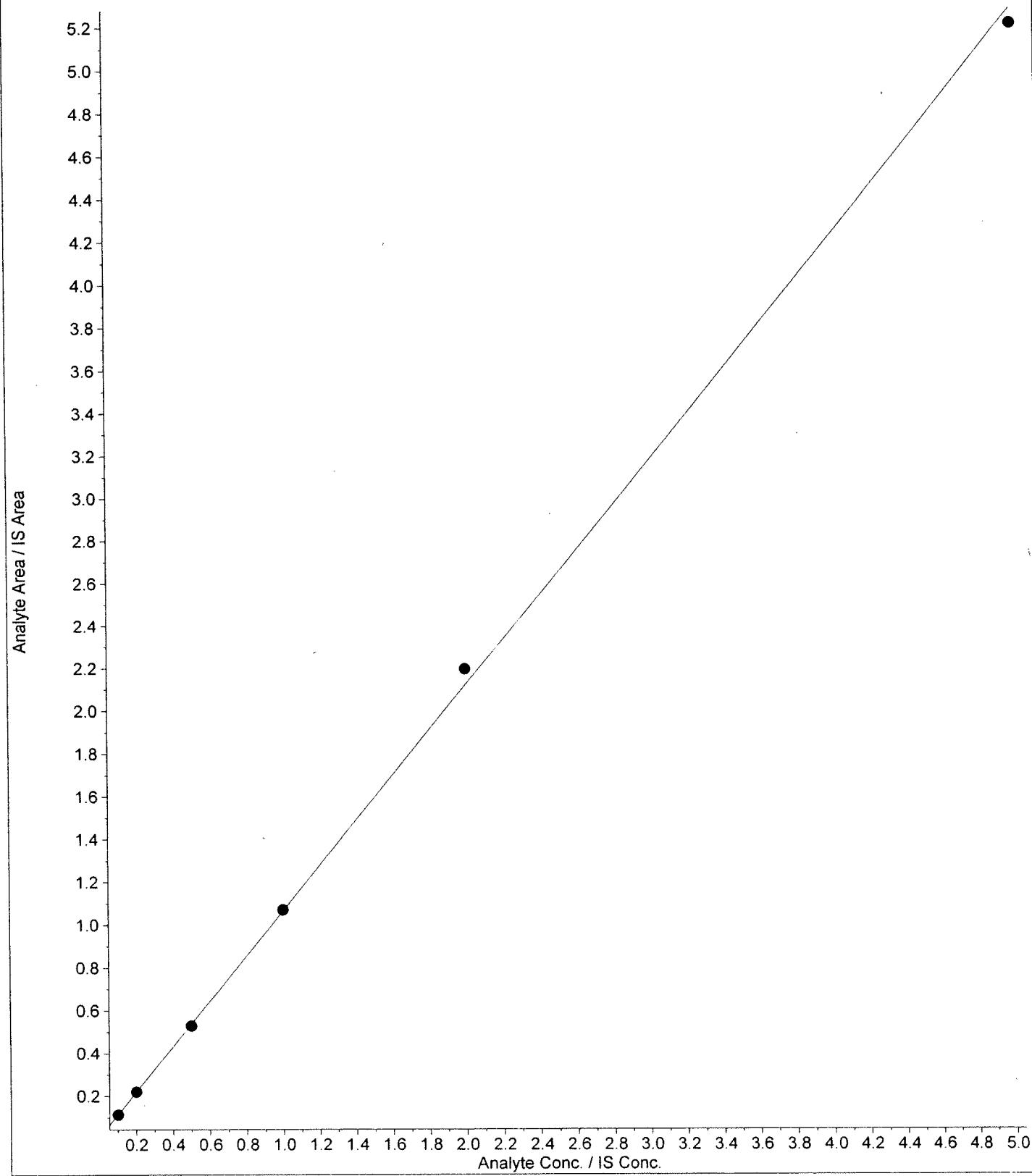
Printing Time: 9:09:26 AM
Printing Date: Wed, Nov 25, 2009
Analyst Version: 1.4.2

MPI Study No.: L19346
Set No.: 112409B

Operator: Mark Neeley
Instrument No.: LC/MS/MS # 9

MDN 11-25-09

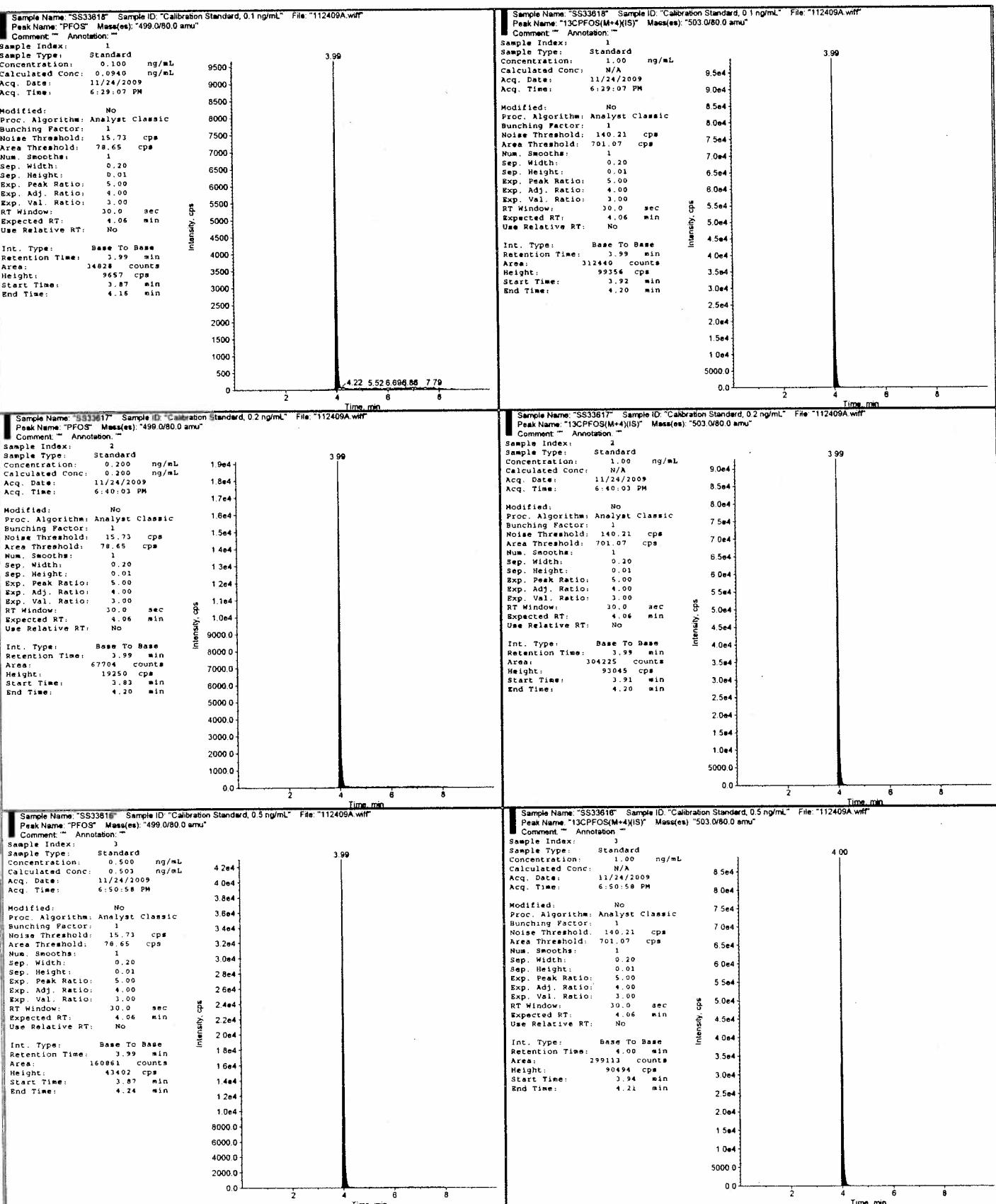
■ Untitled 4 (PFOS): "Linear" Regression ("1 / x" weighting): $y = 1.06 x + 0.00878$ ($r = 0.9997$)



Printing Time: 9:10:18 AM
 Printing Date: Wed, Nov 25, 2009
 Analyst Version: 1.4.2

MPI Study No.: L19346
 Set No.: 112409B

Operator: Mark Neeley
 Instrument No.: LC/MS/MS # 9



MPI Research, Inc.

Initials MN

Page 1 of 5

Date 11-25-09

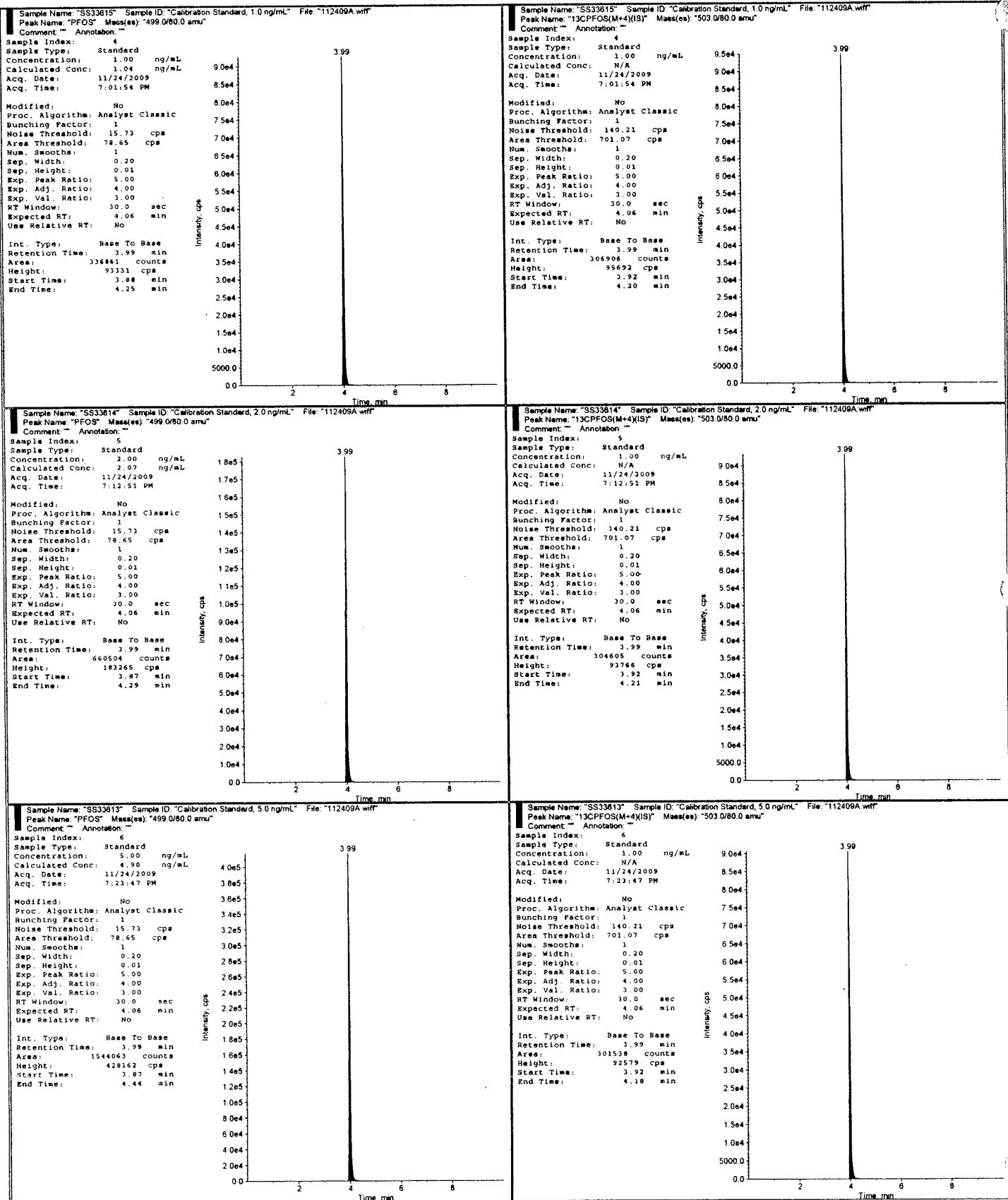
Sample

Index 1 To 15

Printing Time: 9:10:18 AM
 Printing Date: Wed, Nov 25, 2009
 Analyst Version: 1.4.2

MPI Study No.: L19346
 Set No.: 112409B

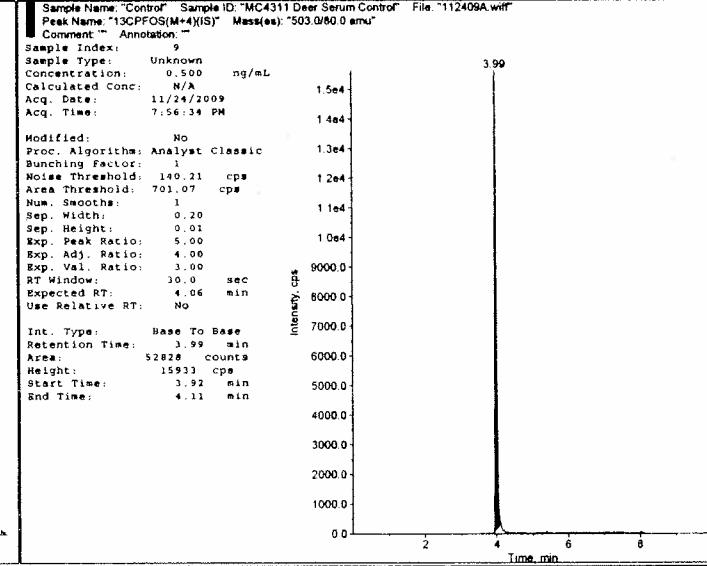
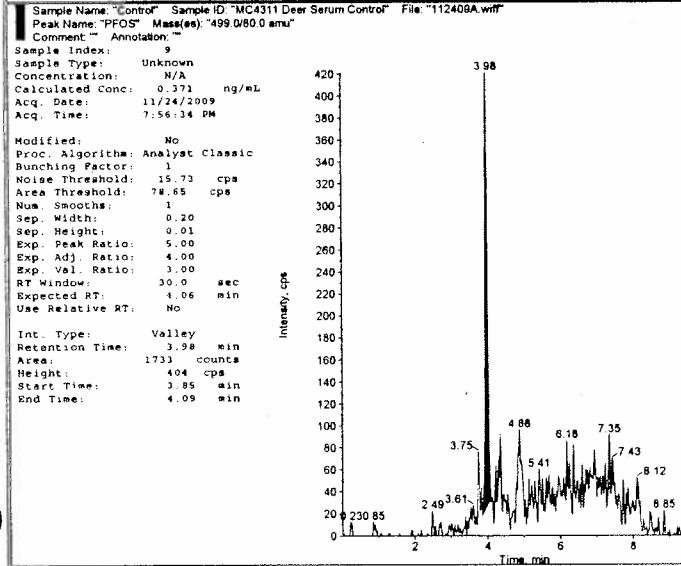
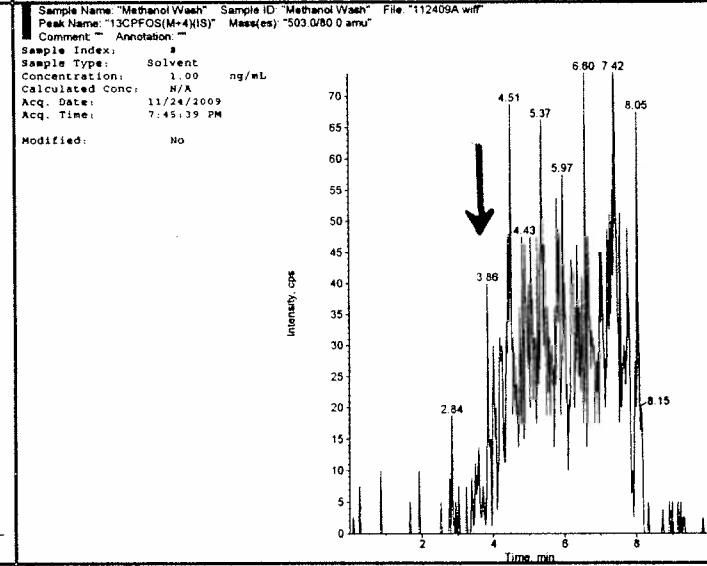
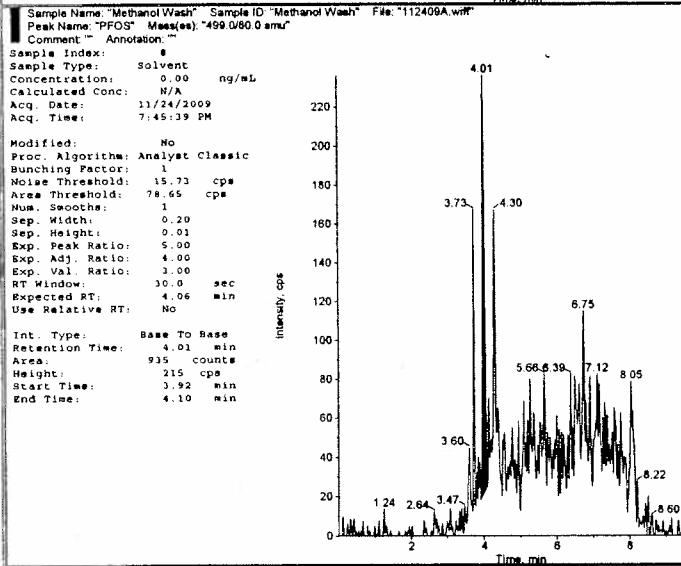
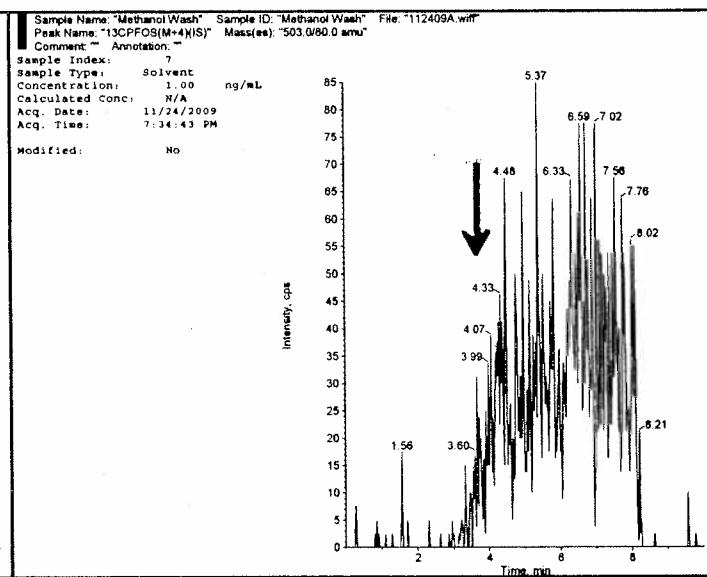
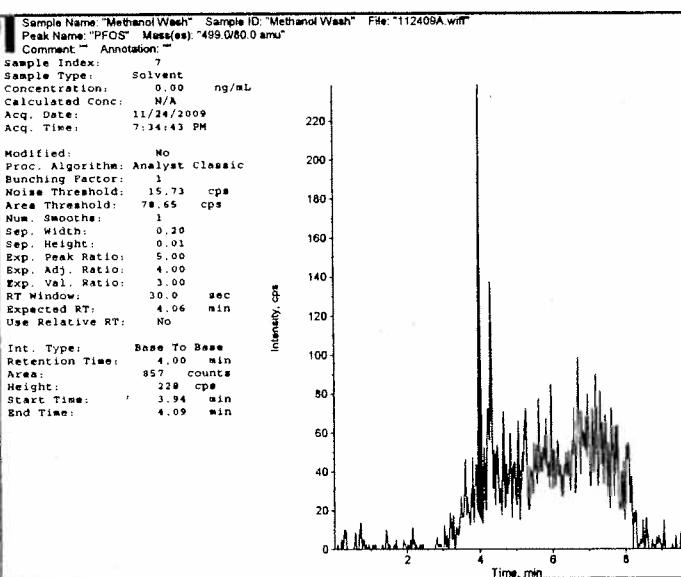
Operator: Mark Neeley
 Instrument No.: LC/MS/MS # 9



Printing Time: 9:10:19 AM
 Printing Date: Wed, Nov 25, 2009
 Analyst Version: 1.4.2

MPI Study No.: L19346
 Set No.: 112409B

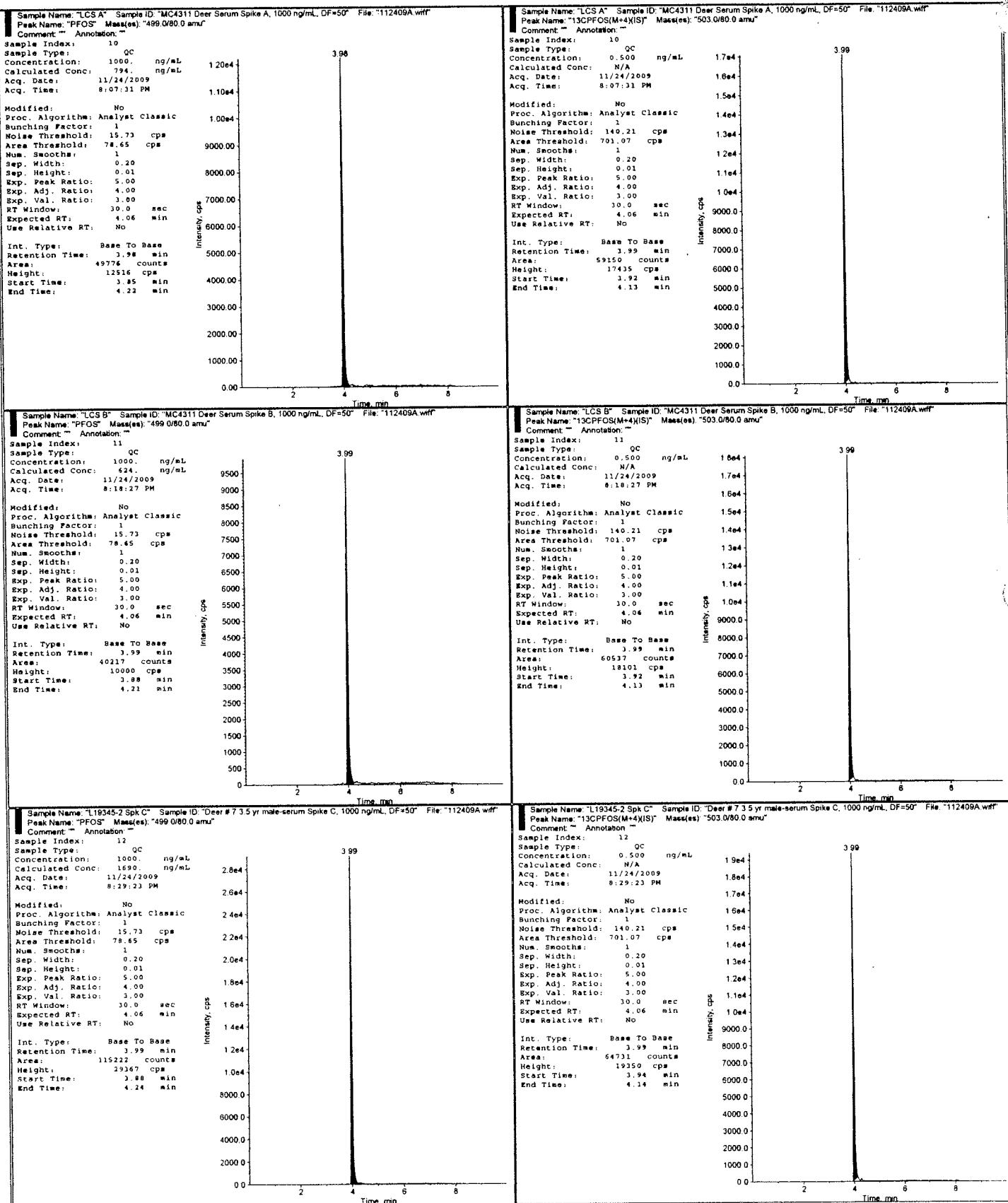
Operator: Mark Neeley
 Instrument No.: LC/MS/MS # 9



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